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Pediatric Preclinical Testing Program (PPTP) – A Molecularly Characterized Panel of Childhood Cancer Models for New Agent Testing

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Abstract

The PPTP is an NCI initiative to address the challenge facing pediatric oncology researchers of reliably prioritizing new agents for study in children with cancer. The PPTP will systematically test 10-15 new agents annually against *in vitro* and *in vivo* preclinical models of childhood cancers, with testing ideally occurring near the time that the agents are entering testing in humans and prior to their initial evaluation in children.

The PPTP's *in vivo* tumor panels include xenograft lines for sarcomas (rhabdomyosarcoma and Ewing sarcoma) (n=8), neuroblastoma (n=6), osteosarcoma (n=6), acute lymphoblastic leukemia (n=8), brain tumors (n=4 glioblastoma, n=6 non-glioblastoma), and kidney cancers (n=6). Stage 1 *in vivo* testing will occur at the tested agent's MTD. Agents will also be tested against the PPTP *in vitro* panel, which includes 23 cell lines representing a variety of childhood cancers. Those agents that demonstrate sufficient activity (either broad-spectrum or histiotype specific) in Stage 1 testing will be considered for Stage 2 testing. Stage 2 testing will define dose response relationships using tumor models in which activity was observed in Stage 1 and will include detailed pharmacokinetic and pharmacodynamic studies to establish the relationship between systemic exposure and antitumor activity. Stage 2 testing may also include evaluation in appropriate secondary models (e.g., orthotopically implanted tumors) to confirm or refute results obtained using subcutaneous tumors. Genetically engineered mouse models may also be utilized when relevant models are available.

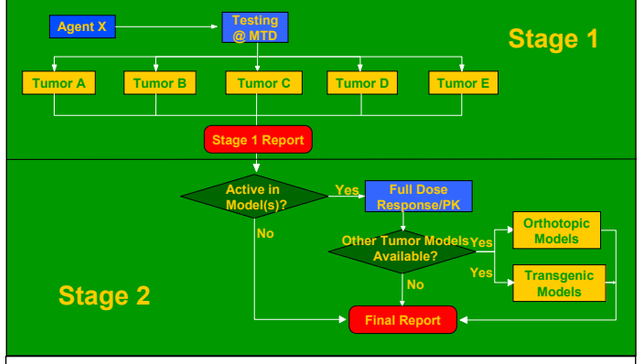
The PPTP xenograft and cell lines are being molecularly characterized, including gene expression profiling using both cDNA and Affymetrix arrays, and LOH and chromosome copy number assessment using the Affymetrix GeneChip 10K array. Tissue arrays and protein lysate arrays will be available for documenting protein expression. Initial gene expression results using cDNA arrays confirm the close relationship between the xenograft models and their respective cancers of origin.

Results obtained by the PPTP will be correlated with the clinical activity and the pharmacokinetic profile of the tested agents in children to assess the predictive capabilities of the PPTP's childhood cancer panels. If successful, the PPTP will markedly facilitate the selection of appropriate agents for clinical evaluation.

Pediatric Preclinical Testing Program

- **NCI-supported research contract for testing new agents using *in vitro* and *in vivo* panels of childhood cancer**
  - **Dr. Peter Houghton – Principal Investigator**
  - **Dr. Malcolm Smith – NCI Project Officer**
- **Testing based on tumor panels representing many of the more common childhood cancers.**
  - **Panels range from 4-8 lines selected to represent the diverse biology and prior therapy of the relevant primary diagnoses.**
  - ***In vivo* panel with 44 xenograft lines (5 test sites)**
  - ***In vitro* panel with 23 cell lines (1 test site)**
  - **Genetically engineered models also available for use in special situations**
- **Plan to test approximately 12 new agents per year against the PPTP childhood cancer panels, with testing initiated April 2005**

PPTP Stage 1 and Stage 2 Testing Schematic



Stage 1 Testing – Efficacy testing at MTD

- **Determine MTD on administration schedule to be used when necessary**
- **Generally test across entire panel**

Stage 2 Testing – May include one or more of the following if clear activity signal observed in Stage 1 testing:

- **Dose-response for selected sensitive lines**
- **Pharmacokinetics**
- **Evaluation of target modulation and other pharmacodynamic endpoints**
- **Combinations (e.g., with standard chemotherapy agents)**

For agents identified as active, comparison of the pharmacokinetic behavior of the agents between mice and humans will be performed to determine whether antitumor activity occurs at drug levels that are achievable in humans.

PPTP Tumor Panels and Test Sites

- **Rhabdomyosarcoma (n=5) and Ewing sarcoma (n=3)**
  - **Dr. Peter Houghton (St. Jude Children's Research Hospital)**
- **Neuroblastoma (n=6)**
  - **Dr. John Maris (Children's Hospital of Philadelphia)**
- **Osteosarcoma (n=6)**
  - **Dr. Richard Gorlick (Albert Einstein College of Medicine)**
- **Acute lymphoblastic leukemia (n=8)**
  - **Dr. Richard Lock (Children's Cancer Institute Australia)**
- **Brain tumors, Glioblastoma (n=4)**
  - **Dr. Henry Friedman (Duke University Medical Center)**
- **Brain tumors, Non-glioblastoma (n=6)**
  - **Dr. Peter Houghton (St. Jude Children's Research Hospital)**
- **Kidney cancers (n=6)**
  - **Dr. Peter Houghton (St. Jude Children's Research Hospital)**
- **In vitro panel (n=23)**
  - **Dr. Patrick Reynolds (Children's Hospital of Los Angeles)**

Selection of Agents for PPTP Testing

***Agent characteristics*:**

- **Plausible relevance to the treatment of childhood cancers**
- **Agent likely to proceed to clinical testing in children within 12-24 months (generally implies agent already in clinic for adults)**
- **Agent's molecular target (or mechanism of action) not previously evaluated by PPTP**
- **Sufficient quantity of agent available for testing across entire PPTP panel**

Pediatric Drug Development Group (PedDDG):

- **PedDDG advises NCI Project Officer on all aspects of PPTP performance**
- **Members are NCI staff with expertise in preclinical and clinical new drug development**
- **Candidate agents for PPTP evaluation reviewed by PedDDG**
- **Candidate agents for PPTP testing should be proposed to the NCI Project Officer**

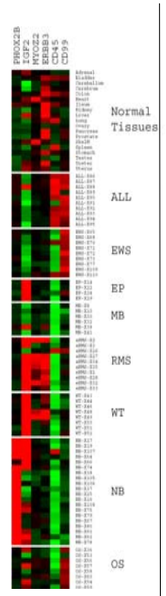
***Priority molecular targets identified for PPTP evaluation*:**

- **Hsp90**
- **Histone deacetylase**
- **Vascular endothelial growth factor receptor**
- **mTOR**
- **Insulin-like growth factor receptor-I**
- **Raf kinase**
- **Proteasome**
- **Src family kinases**
- **Bcl-2 family**

Intellectual Property Issues Relevant to PPTP Agent Testing

- ***Model MTA developed for the PPTP with input from pharmaceutical sponsors and academic institutions*:**
  - **PPTP MTA based on template MTA used for CTEP non-clinical studies, with modifications to accommodate specific needs of PPTP**
  - **PPTP testing sites have all accepted the terms of the model MTA**
- ***Key provisions of MTA include*:**
  - **Company receives non-exclusive royalty free license to any invention for all purposes, including commercial and an option to negotiate an exclusive royalty bearing license.**
  - **In contrast to normal CTEP IP option, company has one year to notify institution of interest in obtaining an exclusive license**
  - **45 day company review of all manuscripts and 10 day abstract review**

Molecular Characterization of Xenografts



***Molecular characterization of xenograft lines*:**

- **Gene expression profiles (cDNA & Affymetrix arrays)**
- **SNP analysis using Affymetrix GeneChip Human Mapping 10K array for quality control and for assessment of LOH & chromosome copy number**
- **CGH using long oligonucleotide microarrays**
- **Tissue arrays for immunohistochemical testing**

***Initial results of molecular characterization studies*:**

- **Expression profiles (cDNA array) currently available for subset of PPTP xenograft lines [See Abstract #4462 (Whiteford, et. al.) for detailed results]**
- **For neuroblastoma, Ewing sarcoma, and rhabdomyosarcoma, xenograft lines identified whose expression profiles are similar to the cancer of origin**
- **For other diagnoses, surrogate measure for similarity is co-clustering within diagnoses**
- **Gene expression results show consistent tumor patterns (see left). For example:**
  - **PHOX2B expression in neuroblastoma**
  - **IGF2 expression in rhabdomyosarcoma and Wilms tumor**
  - **CD99 expression in Ewing sarcoma, with confirmation of protein expression using immunohistochemical methods applied to tissue arrays (not shown)**
- **Searchable database of mRNA and protein expression being built**
- **Affymetrix array data available within 6-9 months**

***Utility of expression array data*:**

- **Identification of xenograft and cell lines expressing targets of interest**
- **Identification/validation of molecular markers predictive of response to tested agent**

Pediatric Preclinical Testing Program (PPTP) - SUMMARY

- **Well-founded prioritization decisions are central to future treatment advances for children with cancer.**
- **The PPTP was established to develop methods for reliably prioritizing new agents for evaluation in children with cancer. The PPTP:**
  - **Builds upon a history of successful clinical correlation & prediction for pediatric preclinical testing,**
  - **Incorporates mouse versus human PK comparisons into prioritization decisions, and**
  - **Will test 10-15 agents per year for their activity across the PPTP's tumor panels.**
- **The PPTP utilizes molecularly characterized preclinical lines, allowing:**
  - **Comparison to primary tumors to assure comparability,**
  - **Identification/validation of molecular markers predictive of response, and**
  - **Identification/validation of relationships between target modulation & antitumor activity.**
- **The PPTP, if successful, can make substantial contributions to the development of more effective treatments for children with cancer through early identification of novel agents likely to be active against one or more childhood cancers.**